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Therapeutic targeting of cancer stem cell-specific surface glycans and glycoproteins

Mutaz Jamal Al-khreisat[1], Waleed K. Abdulsahib[2*], Ihsan Khudhair Jasim[3], H. Malathi[4], Priya Priyadarshini Nayak[5], D. Alex Anand[6], Gunjan Mukherjee[7], Aashna Sinha[8] and Gulsara Ruziyeva[9]

*Correspondence:
Waleed K. Abdulsahib
waleedk.abdulsahib@uoalfarahidi.edu.iq

Full list of author information is available at the end of the article

Abstract

Cancer stem cells (CSCs) represent a critical subpopulation within tumors, driving malignancy, metastasis, and therapeutic resistance. Their unique biological attributes, particularly the distinctive landscape of surface glycans and glycoproteins, offer promising avenues for targeted therapeutic intervention. This comprehensive review explores the pivotal role of CSC-specific surface glycans and glycoproteins, including CD133, CD44 variants, EpCAM, MUC1, gangliosides (GD2, GD3), and truncated O-glycans (Tn, Sialyl Lewis X), as key drivers of CSC properties and therapeutic vulnerabilities. The report details current therapeutic strategies, such as monoclonal antibodies, antibody-drug conjugates, and advanced chimeric antigen receptor (CAR) T, NK, and NKT cell therapies, which are engineered to exploit these surface markers. Furthermore, it examines small molecule inhibitors that target CSC metabolism (metabostemness, the metabolic reprogramming that supports CSC stemness) and induce ferroptosis, a form of regulated cell death characterized by iron-dependent lipid peroxidation, often synergistically. Significant challenges in clinical translation, including achieving specificity, overcoming tumor heterogeneity, modulating the immunosuppressive tumor microenvironment, and addressing manufacturing complexities, are critically discussed. The integration of multi-modal diagnostics, combining multiple diagnostic approaches, encompassing liquid biopsy, advanced imaging, and single-cell epigenomics, is presented as an essential future direction for precision oncology, enabling real-time monitoring and personalized treatment strategies for high-risk cancers like neuroblastoma. This review underscores the imperative for innovative, multi-pronged approaches to effectively eradicate CSCs and achieve durable patient responses.

Keywords Cancer stem cells, Surface glycans, Glycoproteins, Targeted therapy, Precision oncology

1 Introduction

Cancer remains a formidable global health challenge, characterized by its remarkable heterogeneity, metastatic propensity, and resistance to conventional therapies. A critical paradigm shift in understanding tumorigenesis has been the emergence of the cancer stem cell (CSC) hypothesis. CSCs, a small but potent subpopulation within tumors, are

uniquely characterized by their self-renewal capacity, pluripotency, and ability to continuously proliferate, driving tumor initiation, progression, metastasis, and crucially, therapeutic resistance [1]. Their resistance mechanisms allow them to survive treatments, leading to relapse [2].

The cell surface, adorned with a dense and dynamic array of glycans and glycoproteins forming the glycocalyx, represents a critical interface for cellular interactions and signaling within the tumor microenvironment [3]. Aberrant glycosylation, a pervasive hallmark of cancer, involves profound alterations in the structure and expression of these surface carbohydrates and their associated proteins. These changes are not merely epiphenomena; they facilitate malignant behaviors such as enhanced proliferation, invasion, metastasis, and, crucial to CSCs, immune evasion and drug resistance. The unique and often tumor-specific glycosylation patterns on CSC surfaces render them highly attractive and accessible targets for selective therapeutic intervention, offering a promising avenue to overcome the limitations of conventional therapies and achieve lasting remission [4]. This review focuses on CSC-specific surface glycans and glycoproteins, their functional roles, and therapeutic strategies targeting them. The structure is as follows: first, key markers are discussed; then, therapeutic strategies including antibody-based, cellular immunotherapies, small molecule inhibitors, and vaccines; followed by challenges and future directions including integrated diagnostics; and finally, a conclusion.

1.1 Cancer stem cell (CSC) surface glycans and glycoproteins as therapeutic targets

The unique biological properties of cancer stem cells (CSCs), including their self-renewal capacity, tumor-initiating potential, and resistance to conventional therapies, underscore the critical need for targeted therapeutic approaches [5, 6]. A promising avenue lies in exploiting the distinctive landscape of glycans and glycoproteins on the CSC surface [7]. These molecules are not merely passive markers; they actively mediate crucial cellular processes that drive malignancy.

1.2 Key glycan and glycoprotein markers of CSCs

CD133 (Prominin-1).

CD133 is a widely recognized glycosylated transmembrane protein, extensively used as a marker for isolating and identifying CSCs across various cancer types, including brain, colon, pancreas, liver, skin, prostate, and ovarian cancers [8]. Its N-glycosylation is critical for proper protein folding, molecular trafficking, stability, and cell surface localization [9]. CD133-positive cells are associated with CSC properties and poor patient outcomes [10].

The critical role of glycosylation in CD133 function is evident in studies showing that inhibiting N-glycan biosynthesis or its transfer to CD133 results in undetectable cell surface AC133, an important epitope [11, 12]. This suggests that the proper glycosylation of CD133 is indispensable for its presentation on the cell surface. Since CD133 expression is directly linked to the self-renewal [13], tumorigenicity [14], and drug resistance [15] of CSCs, any intervention that disrupts its glycosylation could effectively impair these malignant properties. This understanding highlights that targeting the glycosylation machinery of CD133, rather than just the protein itself, could offer a potent strategy to undermine CSC viability and overcome therapeutic resistance. Additionally, in brain tumors, recent studies support the role of TRPM2 channels in tumor survival and

treatment resistance [16], and TMED2 expression is linked to CSC characteristics [17]. Together, these findings strengthen the argument that brain tumor CSCs rely on ion-channel regulation, trafficking proteins, and altered glycoprotein biology, not just on classical markers such as CD133.

1.3 CD44 and its variants

CD44 is a transmembrane receptor that binds hyaluronic acid and other extracellular matrix components, playing crucial roles in cell-cell and cell-matrix adhesion, epithelial-mesenchymal transition (EMT), and cell proliferation [18]. Notably, CD44, particularly its variant isoforms (e.g., CD44v6), functions as a CSC marker and is a critical player in regulating CSC properties, including self-renewal, tumor initiation, metastasis, and resistance to chemoradiotherapy [8, 19, 20]. Aberrant O-glycosylation of CD44 can significantly alter its binding to ligands, influencing processes like osteoclastogenesis and bone metastasis [21]. Furthermore, specific fucosylation patterns on CD44 have been shown to promote stemness [22].

The glycosylation patterns of CD44 are not merely incidental modifications but significantly modulate its functional engagement with the tumor microenvironment and its contribution to CSC properties. For instance, studies have shown that O-glycosylation near CD44's recognition sites affects its reactivity to antibodies, and that this glycosylation is differentially regulated between tumor and stromal cells [23]. This implies that specific glycoforms of CD44 are uniquely presented on cancer cells. Furthermore, fucosylation of CD44 has been directly linked to increased stemness in mesenchymal stem cells [22]. This indicates that the specific glycosylation profile of CD44 is integral to its ability to promote self-renewal, invasion, and drug resistance. Therefore, targeting the enzymes responsible for these aberrant glycosylation patterns [24], or developing therapies that specifically recognize these altered glyco-epitopes [25], could provide a highly selective approach to disrupt CSC function while minimizing harm to normal cells.

1.4 EpCAM (epithelial cell adhesion molecule)

EpCAM is a type I transmembrane glycoprotein that is frequently overexpressed in various epithelial cancers and stem cells [26]. It plays diverse biological roles, including cell adhesion, migration, proliferation, and differentiation, and is strongly associated with the regulation of cancer stemness [26]. The intracellular domain of EpCAM (EpICD) can be cleaved and transported to the nucleus, where it stimulates gene transcription that promotes cell growth, cancer stem cell properties, and EMT.70 Carcinoma tissues often exhibit hyperglycosylation of EpCAM compared to normal epithelia [26].

EpCAM's multifaceted role in cancer biology, acting as both an adhesion molecule and a signaling mediator, is profoundly influenced by its glycosylation status. The observation that EpCAM is hyperglycosylated in carcinoma tissue compared to normal epithelia suggests a fundamental alteration in its structure that may contribute to its oncogenic functions [26]. This structural change could modulate its interactions with other molecules on the cell surface or influence its cleavage and subsequent nuclear translocation of EpICD, which is known to promote CSC properties and EMT.70 Understanding these glycosylation-dependent functional shifts is crucial. For instance, if specific glycosylation patterns promote the cleavage of EpICD or enhance its nuclear activity, then targeting these modifications could selectively disrupt EpCAM's pro-tumorigenic signaling

without broadly affecting its normal adhesive functions [27]. This approach could offer a refined strategy to target CSCs by exploiting the subtle, yet critical, differences in EpCAM's post-translational modifications.

1.5 Mucin 1 (MUC1)

MUC1 is a transmembrane glycoprotein that is aberrantly glycosylated and overexpressed in a wide variety of epithelial cancers [28]. Unlike normal MUC1, tumor-associated MUC1 (TA-MUC1) often exhibits truncated glycosylation, which exposes cryptic peptide epitopes, making it an attractive target for therapeutic antibodies [28]. MUC1 actively participates in intracellular signal transduction pathways, regulating gene expression and promoting tumor progression [29]. Critically, the MUC1-C subunit has been shown to play a pivotal role in conferring drug resistance in prostate cancer CSCs by upregulating aerobic glycolysis and suppressing reactive oxygen species necessary for self-renewal [30].

The aberrant glycosylation of MUC1 is a defining characteristic of cancer, transforming it into a uniquely tumor-specific target and a driver of therapeutic resistance. In normal cells, MUC1 is heavily glycosylated, shielding its peptide core; however, in cancer, incomplete glycosylation exposes previously hidden peptide epitopes, which are then recognized by antibodies [29]. This structural alteration creates a distinct molecular signature on cancer cells, providing a selective target for therapeutic intervention. Furthermore, the MUC1-C subunit is not merely a marker but actively contributes to drug resistance in prostate cancer CSCs by promoting aerobic glycolysis and suppressing reactive oxygen species, processes essential for CSC self-renewal [30]. This dual role of MUC1—as a source of tumor-specific epitopes due to aberrant glycosylation and as a direct mediator of drug resistance through metabolic reprogramming—underscores its profound importance as a therapeutic target [31]. Strategies that exploit these specific glycoforms could not only lead to the selective elimination of CSCs but also disarm their inherent resistance mechanisms, offering a more comprehensive and durable anti-cancer effect [32].

1.6 Gangliosides (e.g., GD2, GD3)

Gangliosides are a family of glycosphingolipids found on the surface of tumor cells [33]. Disialoganglioside GD2 is highly expressed in neuroblastoma, melanoma, glioma, and various sarcomas, making it a well-established target for immunotherapy [34]. The GD3 ganglioside has been demonstrated to actively promote cell growth, plasticity, and chemotherapy resistance in human glioblastoma CSCs [33]. Importantly, silencing the enzyme responsible for GD3 synthesis (ST8SIA1) leads to decreased CSC properties, reduced migratory capacities, and increased sensitivity to temozolomide chemotherapy, highlighting their functional significance [33].

The functional significance of gangliosides extends beyond their role as mere surface markers; they are active participants in driving CSC malignancy and therapeutic resistance. For instance, GD3 is identified as the main ganglioside in glioblastoma, with strong expression in patient-derived cancer stem-like cells [33]. The reduction of GD3-positive populations upon cell differentiation further supports its association with stemness [35]. Evidence supporting a functional link to malignancy is demonstrated by studies where silencing ST8SIA1, the enzyme for GD3 synthesis, leads to a significant

decrease in CSC properties such as sphere size and self-renewal, reduced migratory capacity, and crucially, increased sensitivity to temozolomide chemotherapy [33]. This indicates that targeting gangliosides is not simply about identifying CSCs, but rather about directly disrupting their ability to proliferate, invade, and resist chemotherapy [36]. This mechanistic understanding provides a strong rationale for developing therapies that specifically interfere with ganglioside biosynthesis or function to overcome CSC-mediated resistance.

1.7 Truncated O-Glycans (e.g., Tn, Sialyl Tn, Lewis Y, Sialyl Lewis X)

Truncated O-glycans, such as the Tn antigen (GalNAcα1-O-Ser/Thr) and its sialylated version, Sialyl Tn (STn), are commonly expressed in a majority of human carcinomas due to a blockage in the normal O-glycosylation pathway [37]. Their expression correlates with metastatic potential and poor prognosis in many cancers, including breast, gastric, colorectal, and lung carcinomas [38]. Similarly, Sialyl Lewis X (sLex) and Sialyl Lewis A (sLea) are tetrasaccharide structures that serve as ligands for selectins, promoting cancer cell adhesion to endothelium and facilitating metastatic dissemination [38]. These glycans also influence immune recognition and angiogenesis [39].

The presence of truncated O-glycans and specific Lewis antigens on the surface of cancer cells is a direct consequence of altered glycosylation machinery in malignancy, and these aberrant structures facilitate tumor progression and immune evasion. The Tn antigen, for example, arises from a blockage in normal O-glycosylation, leading to its abnormal expression in cancer.26 This structural deviation is not benign; it is strongly correlated with increased metastatic potential and poor patient outcomes [40]. Similarly, sLex and sLea, by acting as ligands for E-selectin, facilitate the critical step of cancer cell adhesion to endothelial cells, enabling metastatic spread [39]. Furthermore, hypersialylation, which includes sLex expression, promotes tumor metastasis by enhancing immune evasion and stimulating tumor invasion [41]. This indicates that these aberrant glycans are not merely bystanders but active participants in the metastatic cascade and immune escape [42]. Targeting these specific, abnormally expressed glycan structures or the enzymes that produce them offers a direct approach to disrupt multiple pro-tumorigenic pathways, potentially inhibiting metastasis and sensitizing CSCs to immune attack [43].

1.8 Glycoprotein NMB (GPNMB)

Glycoprotein NMB (GPNMB) is a type I transmembrane protein that is highly expressed in various cancers, including breast cancer, glioblastoma, and lung cancer, and its high expression is often associated with poor prognosis [44]. GPNMB promotes tumor growth, invasion, and metastasis, and has been shown to induce stem cell-like properties in breast epithelial cells [44]. Notably, GPNMB expression is often observed in growth-arrested, dormant cancer cells, and its tumorigenic activity is dependent on serine phosphorylation within its intracellular domain [44].

The role of GPNMB in promoting stemness and its association with quiescent cancer cells highlights its critical contribution to tumor relapse and therapeutic resistance [45]. GPNMB's ability to induce sphere formation and tumor growth, coupled with its presence in dormant cancer cells, suggests it plays a key role in maintaining the CSC reservoir that survives conventional therapies [44]. The observation that its tumorigenic

activity is dependent on serine phosphorylation further points to specific signaling pathways that could be targeted [46]. This indicates that GPNMB is not simply a marker of aggressive disease but an active mediator of CSC properties, particularly their ability to persist in a quiescent state and re-initiate tumor growth [45]. Therefore, therapeutic strategies aimed at inhibiting GPNMB's function, perhaps by blocking its phosphorylation or its interaction with growth factor receptors like EGFR [47], could effectively eliminate these dormant, therapy-resistant CSCs and prevent tumor recurrence [47] (Fig. 1).

1.9 Therapeutic strategies targeting CSC-specific surface glycans and glycoproteins

The unique molecular signatures presented by CSC-specific surface glycans and glycoproteins provide a compelling foundation for the development of highly targeted therapeutic interventions. These strategies aim to selectively eliminate CSCs, overcome their inherent resistance mechanisms, and ultimately prevent tumor relapse and metastasis.

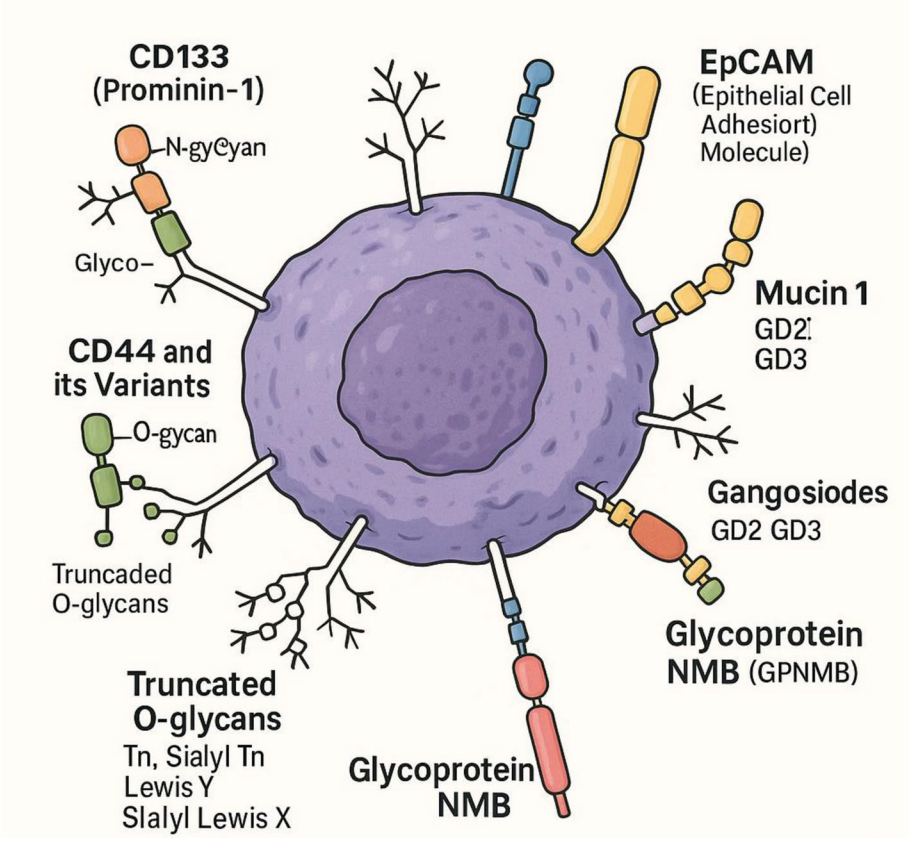

Fig. 1 Key glycans and glycoproteins on CSC surfaces, including CD133 (N-glycosylated transmembrane protein), CD44 and its variants (with O-glycosylation and fucosylation patterns), EpCAM (hyperglycosylated adhesion/signaling protein), MUC1 (aberrantly glycosylated transmembrane glycoprotein), gangliosides GD2 and GD3 (glycosphingolipids driving CSC malignancy), truncated O-glycans (Tn, Sialyl Tn, Lewis Y, Sialyl Lewis X, promoting metastasis and immune evasion), and Glycoprotein NMB (GPNMB, linked to CSC quiescence and therapeutic resistance). These molecules regulate self-renewal, invasion, immune evasion, and drug resistance, providing promising avenues for targeted therapeutic interventions

1.10 Antibody-Based therapies

1.10.1 Monoclonal antibodies (mAbs)

Monoclonal antibodies (mAbs) represent a well-established class of therapeutics that can directly target CSC-specific surface glycans and glycoproteins [48]. These antibodies are designed to bind with high affinity and specificity to unique or overexpressed antigens on tumor cells, leveraging the host's immune system to mediate anti-tumor effects [49, 50]. Examples of targets include gangliosides like GD2 and GD3, glycoproteins such as EpCAM and MUC1, and carbohydrate antigens like Lewis Y and Sialyl Lewis X, as well as CD133 [51].

The mechanisms of action for these mAbs include antibody-dependent cell-mediated cytotoxicity (ADCC) [52], complement-dependent cytotoxicity (CDC), and direct induction of cell death or inhibition of signaling pathways [53, 54]. For instance, anti-GD2 antibodies are now a standard of care for high-risk metastatic neuroblastoma, demonstrating significant improvements in overall survival when combined with cytokines [34]. This success underscores the principle that mAbs targeting CSC-specific surface molecules can effectively recruit and activate endogenous immune cells to selectively eliminate CSCs. This targeted approach is crucial for overcoming the inherent resistance and heterogeneity of CSCs, as it provides a precise mechanism to eradicate the root cause of tumor recurrence. By focusing on surface-accessible, tumor-specific targets, mAbs offer a refined therapeutic modality that harnesses the body's own defense mechanisms against the most aggressive cancer cell populations.

1.10.2 Antibody-drug conjugates (ADCs)

Antibody-drug conjugates (ADCs) represent a sophisticated evolution of antibody-based therapies, functioning as targeted drug delivery systems. These engineered molecules comprise a monoclonal antibody linked to a highly potent cytotoxic agent (payload) via a stable chemical linker [55]. The design allows the ADC to specifically bind to tumor-associated antigens on CSCs, such as EpCAM, MUC1, CD44v6, GD2, and Globo H [56, 57]. Upon binding, the ADC-antigen complex is internalized via receptor-mediated endocytosis, leading to the intracellular release of the cytotoxic drug preferentially within the tumor cell [55].

The primary advantage of ADCs lies in their ability to overcome the systemic toxicity often associated with conventional chemotherapy by delivering highly potent cytotoxic agents directly to cancer cells. This targeted delivery mechanism significantly enhances the therapeutic index, meaning higher drug concentrations can be achieved at the tumor site while minimizing off-target effects on healthy tissues [58]. For CSCs, which are notoriously resistant to conventional therapies, this precision is paramount. For example, targeting MUC1-C with an ADC has shown high effectiveness in suppressing the self-renewal of drug-resistant prostate cancer CSCs [30]. Furthermore, recent 2024 studies utilizing nanobody-based delivery systems targeting MUC1 have shown enhanced penetration into dense CSC spheroids compared to conventional antibodies [59, 60]. Recent reviews highlight the potential of CSC-targeted ADCs for cancer immunotherapy [61]. This indicates that ADCs can not only selectively eliminate CSCs but also effectively disarm their intrinsic resistance mechanisms by delivering a lethal payload directly to these elusive cells [62]. The capacity of ADCs to combine the specificity of antibodies with the potency of cytotoxic drugs makes them a powerful tool in the

arsenal against CSCs, offering a promising strategy for more effective and less toxic cancer treatments [63]. Additionally, tumor-targeting nanoplatforms that combine imaging with PDT/PTT/CDT have been developed [64], and studies reinforce relevance in ovarian tumor immunotherapy [65]. Both strengthen the position on multimodal, tumor-microenvironment–responsive platforms targeting CSCs.

1.11 Cellular immunotherapies

1.11.1 Chimeric antigen receptor (CAR) T-cell therapy

CAR T-cell therapy has revolutionized the treatment of hematological malignancies, demonstrating remarkable success in relapsed/refractory B-cell lymphomas and leukemias [66]. Developing CARs against surface glycans presents distinct bioengineering challenges compared to protein targets. Carbohydrate antigens often elicit low-affinity antibody responses, making the isolation of high-affinity single-chain variable fragments (scFvs) technically demanding. Unlike protein epitopes, glycan-binding scFvs often require precise affinity tuning to distinguish between high-density expression on CSCs and low-level physiological expression on normal tissues, a critical step to prevent on-target/off-tumor toxicity [67]. Furthermore, the flexibility of glycan chains can hinder the formation of a stable immune synapse, necessitating optimized hinge designs to ensure robust T-cell activation.

1.11.2 CSC-Specific targets

CAR T-cells are being developed to target various CSC surface markers, including ALDH1A1 183 [68], CD133 184 [69], GD2 186 [36], EpCAM 38 [70], LGR5 191 [71], B7-H3 39 [72], GPC3 194 [73], CD44v6 196 [74], PSCA 164, CD166 38 [75], and CD117 [69]. Preclinical studies have shown promising anti-tumor effects by targeting these CSC-associated antigens [18](Fig. 2). Recent clinical data (2023) from a Phase 1/2 trial demonstrated that GD2-directed CAR T-cells were feasible and safe in children with high-risk neuroblastoma, showing objective responses in patients with heavy tumor burdens [76].

1.12 Generic challenges and solutions in CAR T-cell therapy

The immunosuppressive TME, tumor heterogeneity, and limited persistence pose major barriers to CAR T-cell efficacy against CSCs [77]. To address these, strategies include TME modulation through cyclophosphamide pre-conditioning or engineering CAR T-cells to secrete pro-inflammatory cytokines like IL-12 or IL-7 [78]. For antigen escape, dual-targeting or logic-gated CARs enhance specificity and reduce toxicity [79, 80]. Enhancing persistence involves co-stimulatory domains like 4-1BB and alternative cell sources like iPSCs [81]. Furthermore, memory T-cell programming is central to adoptive therapy efficacy, as T-cell memory and TME constraints jointly determine CAR efficacy against CSCs [82]. The 'Glyco-Immune Checkpoint' in CSCs, involving hypersialylation and Siglec binding, requires disruption via sialidases [83]. These approaches aim to sustain anti-tumor responses.

1.13 CAR NK/NKT cell therapy

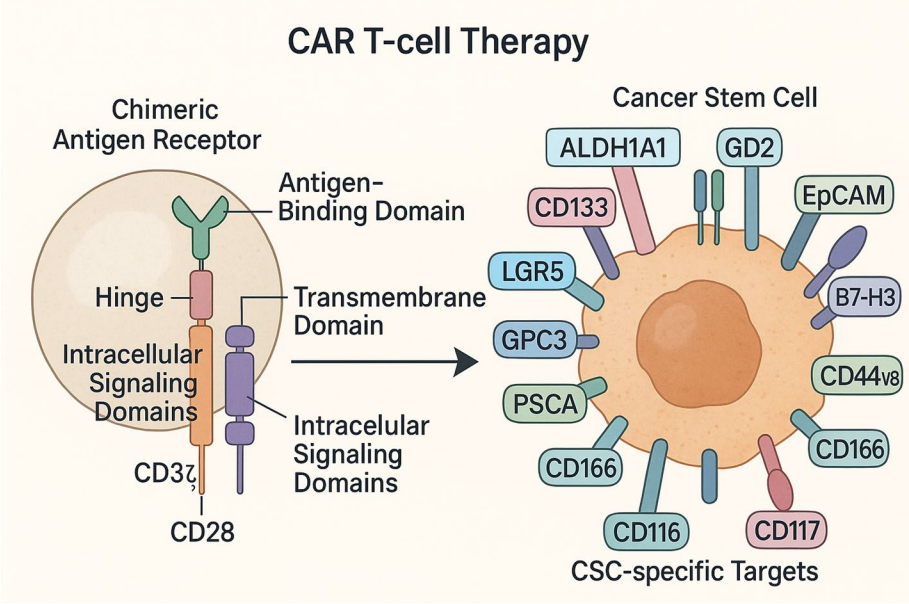

Fig. 2 Structural components of a CAR, including the antigen-binding domain (scFv), hinge region, transmembrane domain, and intracellular signaling modules (CD3ζ, CD28). CSC-specific targets include ALDH1A1, CD133, GD2, EpCAM, LGR5, B7-H3, GPC3, CD44v6, PSCA, CD166, and CD117. The recognition and targeting of CSC-associated surface antigens by CAR T-cells illustrate their therapeutic potential in eradicating CSC populations, while addressing challenges such as antigen heterogeneity, specificity, and immune escape

- Chimeric antigen receptor (CAR) Natural Killer (NK) and Natural Killer T (NKT) cell therapies have emerged as promising alternatives to CAR T-cells, particularly for targeting CSCs.
- Advantages over CAR T-cells: CAR NK/NKT cells offer several key advantages, including a reduced risk of severe cytokine release syndrome (CRS) and neurotoxicity, and a lower incidence of graft-versus-host disease (GvHD) in allogeneic settings, making them safer "off-the-shelf" products [84]. They possess multiple mechanisms for activating cytotoxic activity, including CAR-dependent and natural cytotoxic pathways, and can eliminate target cells without MHC restriction [85]. This inherent versatility allows them to overcome some of the limitations faced by CAR T-cells, such as tumor escape due to CAR antigen loss [86]. The ability to source these cells from allogeneic donors simplifies manufacturing and expands accessibility, addressing critical bottlenecks in CAR T-cell therapy. This means that CAR NK/NKT cells could provide a more readily available and safer cellular immunotherapy platform, particularly valuable for patients who may not be eligible for autologous CAR T-cell therapy or who require rapid treatment [87].
- Current Status and Challenges: While preclinical data for CAR NK/NKT cells are encouraging, particularly in solid tumors, their clinical approval remains limited [84]. Challenges include limited.
- in vivo persistence, lower transduction and expansion efficiencies compared to T-cells, and the need for larger controlled trials to confirm safety and efficacy [84].
- CSC-Specific Targets: CAR NK/NKT cells are being developed to target CSC-associated antigens such as GD2 (neuroblastoma), CD44 (ovarian cancer) 39, and EpCAM [84]. Preclinical studies have demonstrated their potential to preferentially kill CSCs from various human cancer cell lines and primary specimens [88] (Fig. 3).

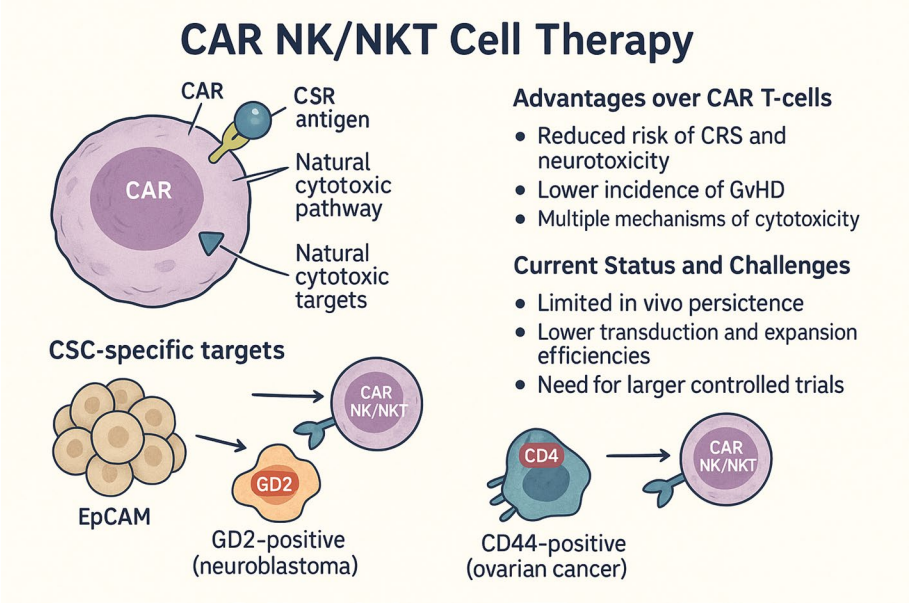

Fig. 3 CAR-engineered NK/NKT cells combining natural cytotoxic pathways with engineered CAR specificity. CSC-specific targets include EpCAM, GD2 (notably in neuroblastoma), and CD44 (e.g., in ovarian cancer). Advantages over CAR T-cells include reduced risk of cytokine release syndrome (CRS) and neurotoxicity, lower incidence of graft-versus-host disease (GvHD), and multiple cytotoxic mechanisms. Current challenges include limited in vivo persistence, lower transduction/expansion efficiencies, and the need for larger controlled trials.**Small Molecule Inhibitors**

1.14 Targeting CSC metabolism (metabostemness)

Cancer stem cells exhibit a remarkable metabolic plasticity, adapting their metabolic programs to survive and proliferate in the harsh tumor microenvironment [89]. This unique metabolic profile, termed "metabostemness," refers to the metabolic parameters that causally control or functionally substitute for the epigenetic reprogramming driving CSC states [90]. CSCs often reprogram their metabolism, shifting between glycolysis and oxidative phosphorylation (OXPHOS), and altering lipid and amino acid metabolism to support tumorigenesis, survival, and drug resistance [1].

Targeting CSC metabolism offers a promising strategy to selectively eliminate these resistant cells by exploiting their unique metabolic vulnerabilities. While general metabolic inhibition is effective, targeting the Hexosamine Biosynthetic Pathway (HBP) is particularly lethal to CSCs. The HBP is the metabolic engine that produces UDP-GlcNAc, the donor substrate required for N-glycosylation and O-GlcNAcylation of key stemness factors like Sox2 and Oct4 [91]. Inhibitors of GFAT, the rate-limiting enzyme of HBP, may reduce cell surface expression of CD133 and CD44, potentially disrupting the CSC glycocalyx and enhancing immune surveillance. By disrupting these critical metabolic pathways, small molecule inhibitors can selectively starve or functionally impair CSCs, thereby reducing their ability to initiate and maintain tumor growth, and importantly, overcome their resistance to conventional therapies. Furthermore, NF-κB inhibition suppresses proliferation in OSCC cell lines [92], while AP-1 signaling controls key oncogenic programs via multi-omics [93]. Together, these strengthen the claims that NF-κB and AP-1 signaling networks drive CSC survival and therapy resistance.

1.15 Glycan modulating agents

1.15.1 Glycosyltransferase inhibitors

Glycosyltransferases are enzymes responsible for synthesizing the complex glycan structures on cell surfaces. Aberrant expression or activity of these enzymes leads to the altered glycosylation patterns characteristic of cancer, including CSCs [3]. Inhibiting specific glycosyltransferases can therefore alter CSC surface glycan profiles, thereby disrupting their pro-tumorigenic signaling, immune evasion, and drug resistance. For example, fucosyltransferase 8 (FUT8) mediates the core fucosylation of B7-H3, an immune checkpoint molecule [94]. Inhibiting FUT8 can lead to defucosylation and lysosomal degradation of B7-H3, exhibiting potent anti-metastatic colorectal cancer activity [94]. This suggests that by modulating the glycan landscape, it is possible to directly impact the functional properties of CSCs and their ability to interact with the immune system. This approach offers a highly specific intervention at the enzymatic level, potentially leading to therapies with reduced off-target effects compared to broader cytotoxic agents.

1.15.2 Glycan-Binding protein (lectin) inhibitors

Glycan-binding proteins, or lectins, mediate crucial interactions between cells and their microenvironment by recognizing specific glycan structures. In cancer, these interactions can promote tumor progression and immune evasion [95]. Targeting these glycan-lectin interactions offers a strategy to disrupt the communication networks that support CSC survival and immune escape. For example, Siglecs (sialic acid-binding immunoglobulin-like lectins) on immune cells can bind to sialylated glycans on cancer cells, inhibiting anti-tumor immune responses [41]. Similarly, galectins (e.g., Galectin-3) play roles in tumor progression and fibrosis [96]. Inhibitors targeting Siglec-15 have shown cytotoxicity in colorectal cancer cells [95]. By blocking these interactions, it may be possible to re-sensitize CSCs to immune attack and restore effective anti-tumor immunity. This strategy focuses on disrupting critical cell-surface communication points, thereby undermining the ability of CSCs to manipulate their surroundings for survival and proliferation.

1.15.3 Metabolic glycoengineering (MGE)

Metabolic glycoengineering (MGE) is a non-genetic strategy that allows for the remodeling of cell surface glycan structures by introducing unnatural monosaccharides [97]. These modified sugars are incorporated into cellular glycosylation pathways, leading to the display of novel glycans on the cell surface [97]. This technique provides an opportunity to reshape the cellular microenvironment and evoke desired cellular responses.

MGE offers a unique approach to alter CSC surface glycans, potentially making them more susceptible to immune attack or less capable of self-renewal, although data in the CSC context remain limited.By presenting unnatural glycans, MGE could disrupt the normal pro-tumorigenic interactions mediated by endogenous glycans, or introduce novel epitopes that can be recognized by the immune system. This non-genetic intervention avoids the complexities and potential risks associated with direct genetic modifications, offering a flexible platform for manipulating CSC phenotypes. The ability to precisely control the glycan landscape on CSCs could lead to innovative therapeutic

strategies that disarm their malignant properties and enhance their vulnerability to existing or emerging treatments.

1.16 Glycan-based vaccines

Glycan-based vaccines aim to activate the immune system against tumor-associated carbohydrate antigens (TACAs) that are aberrantly expressed on cancer cells, including CSCs [98]. These TACAs, such as Tn, Sialyl Tn, GD2, Globo H, MUC1, Lewis Y, and Sialyl Lewis X, are often absent or expressed at very low levels on normal tissues, making them ideal tumor-specific targets [98]. The goal is to elicit a robust and specific immune response (both humoral and cellular) that can recognize and eliminate cancer cells expressing these altered glycans. Glycan-based vaccines may offer a promising prophylactic or therapeutic strategy against CSCs, although actual data on CSC-specific eradication by glycan vaccines are sparse and mainly derive from general TACA-vaccine work in cancer. For instance, vaccines targeting the Tn-MUC1 glycopeptide epitope have shown promising anti-tumor activity in preclinical models [99]. Similarly, clinical trials for Globo H vaccines have shown the induction of anti-Globo H antibodies and correlations with progression-free survival in metastatic breast cancer patients [100]. The challenge lies in overcoming the low immunogenicity of some carbohydrate antigens and ensuring a durable immune response that can effectively target heterogeneous tumor populations. However, by focusing on these unique glycan signatures, vaccines can train the immune system to specifically identify and attack CSCs, potentially preventing recurrence and improving long-term outcomes (Fig. 4).

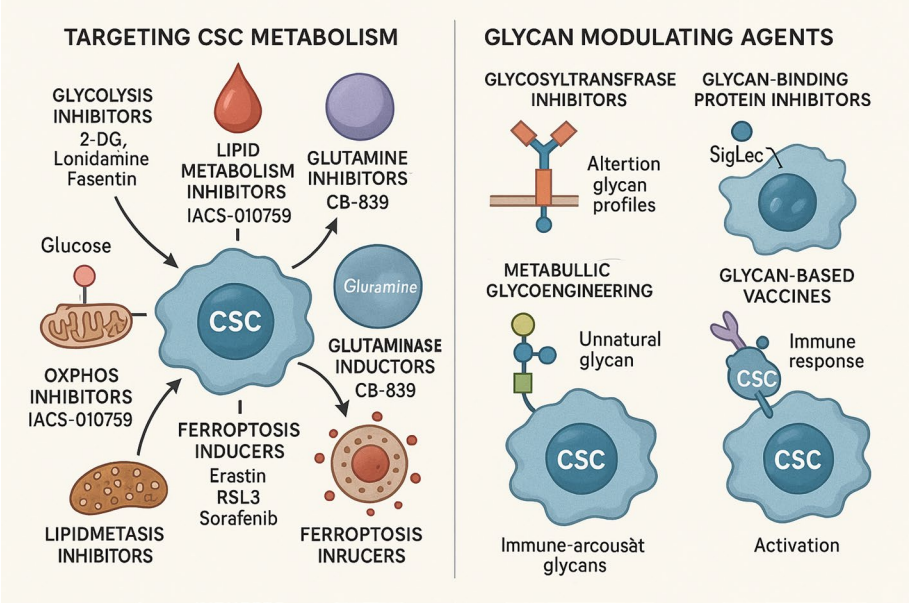

Fig. 4 Therapeutic strategies targeting cancer stem cell (CSC) metabolism and surface glycans. Left panel (Targeting CSC Metabolism): CSCs rely on multiple metabolic pathways that can be selectively inhibited, including glycolysis inhibitors (2-DG, lonidamine, fasentin), lipid metabolism inhibitors (IACS-010759), OXPHOS inhibitors (IACS-010759), glutamine inhibitors and glutaminase modulators (CB-839), and ferroptosis inducers (erastin, RSL3, sorafenib). Right panel (Glycan Modulating Agents): Aberrant CSC surface glycans can be targeted via glycosyltransferase inhibitors, glycan-binding protein inhibitors (e.g., Siglec blockade), metabolic glycoengineering. Table 1: Summary of Key Therapeutic Strategies Targeting CSC Surface Glycans/Glycoproteins and their Clinical Development Status

Table 1 Shows glycan-based vaccines against CSC-specific epitopes

Target Antigen	Therapeutic Modality	Drug/Candidate Name	Mechanism of Action	Evidence Level	Clinical Status / Trial Ref (Year)
GD2	CAR-T Cell	GD2-CART01	Cytotoxicity against GD2 + neuroblasts	Clinical	Phase I/II [76]
GD2	Monoclonal Antibody	Dinutuximab beta	ADCC/CDC immune recruitment	Clinical	Approved (Maintenance for high-risk neuroblastoma) [101]
MUC1-C	ADC (Antibody-Drug Conjugate)	LM-301 (all-MUC1)	Internalization & payload delivery	Pre-clinical / Early Clinical	Phase I [30]
Claudin18.2	CAR-T Cell	CT041	Targeting gastric CSCs/tumor cells	Clinical	Phase Ib/II [102]
CD44v6	CAR-T Cell	CD44v6 CAR-T	Targeting hyaluronic acid binding region	Clinical	Phase I/II [103]
EpCAM	Bispecific Antibody (BiTE)	Solitomab	T-cell engager (EpCAM x CD3)	Clinical	Phase I [104]
Fucosyl-GM1	Monoclonal Antibody	BMS-986,012	Targeted ADCC	Clinical	Phase II [105]
Siglec-15	Monoclonal Antibody	NC318	Blocks immune suppression (Glyco-checkpoint)	Clinical	Phase I/II [106]

1.16.1 Challenges and future directions

Despite significant advancements in targeting CSC-specific surface glycans and glycoproteins, several formidable challenges must be addressed to translate these promising strategies into widespread clinical success.

1.17 Specificity and on-target/off-tumor toxicity

A major hurdle is the challenge of achieving absolute tumor specificity, as many CSC-associated surface antigens are also expressed, albeit often at lower levels, on normal stem cells or healthy tissues [107]. This shared expression can lead to severe on-target/off-tumor toxicities, such as prolonged myeloablation with CD33-targeted therapies or gut and liver toxicities with LGR5-targeting agents [108]. For instance, early anti-CAIX CAR T-cell trials faced serious on-target/off-tumor effects due to CAIX expression on healthy bile ducts [67]. Furthermore, a unique challenge in glyco-targeting is the ubiquitous expression of lectins (carbohydrate-binding proteins) on endothelial cells and liver hepatocytes (e.g., the asialoglycoprotein receptor) [109, 110]. Therapeutic antibodies targeting specific glycans must be engineered to avoid rapid clearance by the liver's innate glycan-clearing mechanisms, a hurdle not present in protein-only targeting [111].

Achieving true CSC specificity while avoiding harm to normal tissues is paramount for the safe and effective application of these therapies. This necessitates sophisticated engineering and a deeper understanding of the subtle quantitative and qualitative differences in antigen expression between healthy and malignant cells. Strategies to mitigate this include:

- Logic-gated CARs: These advanced CAR designs require co-expression of multiple antigens for T-cell activation, significantly enhancing specificity and reducing off-tumor toxicity [81]. For example, LogiCAR designer uses single-cell transcriptomics

to identify cancer-specific antigen circuits with "AND," "OR," and "NOT" logic gates, showing enhanced tumor-targeting efficacy and improved safety profiles [83, 112].

- Affinity Fine-tuning: Engineering CARs or antibodies with lower affinity for antigens expressed at physiological levels on normal cells, but high affinity for the same antigen when highly expressed on tumor cells, can create a wider therapeutic window [67].
- Dual-targeting: Employing two distinct CARs or antibodies against different CSC antigens can provide redundancy and reduce the likelihood of off-target effects while maintaining efficacy against heterogeneous tumors [113].

These approaches represent a concerted effort to move beyond simple antigen presence to a nuanced recognition of tumor-specific molecular signatures, thereby improving the safety profile of CSC-targeted therapies.

1.17.1 Tumor heterogeneity and antigen escape

Tumors are highly heterogeneous, comprising diverse cell populations, including CSCs, which exhibit significant plasticity [1]. This inherent heterogeneity, coupled with the ability of CSCs to undergo phenotypic transitions like EMT, allows for antigen downregulation or loss, leading to therapeutic resistance and antigen escape [114]. For instance, patients treated with CD19-targeted CAR T-cells can relapse with CD19-negative disease due to antigen loss [80].

The plasticity of CSCs and the dynamic nature of tumor heterogeneity necessitate adaptive therapeutic strategies to prevent the emergence of resistant clones. This requires a shift from targeting single antigens to more robust, multi-pronged approaches:

- Multi-antigen targeting: As discussed, dual-targeting CARs or ADCs can simultaneously engage multiple CSC-specific antigens, making it significantly harder for tumors to escape by losing a single target [80].
- Logic-gated CARs: These sophisticated designs provide a built-in mechanism to enhance specificity and reduce antigen escape by requiring complex antigen recognition patterns for activation [81].
- iPSC-derived CARs: Utilizing induced pluripotent stem cells (iPSCs) as a source for CAR T-cells or other immune effectors allows for multiple genetic modifications, potentially enabling the creation of CARs that are less susceptible to antigen escape or can target a broader range of tumor cells [115].

These strategies aim to stay ahead of tumor evolution by building in redundancy and conditional activation, thereby maintaining effective therapeutic pressure on the diverse CSC populations.

1.18 Immunosuppressive tumor microenvironment (TME)

The tumor microenvironment (TME) in solid tumors is a formidable barrier to effective immunotherapy, including CAR-based therapies [116, 117]. It is characterized by physical barriers, a hostile metabolic milieu (hypoxia, low pH, nutrient deprivation), and the presence of immunosuppressive cells (e.g., tumor-associated macrophages (TAMs),

myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs)) and inhibitory molecules (e.g., TGF-β, IL-10, PD-L1, CTLA-4) [4]. These factors collectively impede CAR T-cell infiltration, persistence, and effector function, significantly limiting their efficacy in solid tumors [118].

The TME is a major impediment to effective CSC targeting, requiring multifaceted strategies to remodel it and enhance immune effector function. This involves not just killing tumor cells but reshaping the microenvironment to favor immune attack. Current strategies to overcome the immunosuppressive TME include:

- Armored CARs: Engineering CAR T-cells to secrete pro-inflammatory cytokines (e.g., IL-12, IL-7) or express cytokine receptors (e.g., hybrid IL-7/IL-2 receptors) to modulate the local cytokine milieu and promote T-cell function [78].
- Immune Checkpoint Blockade: Combining CAR T-cell therapy with checkpoint inhibitors (e.g., anti-PD-1/PD-L1, anti-CTLA-4 antibodies) or engineering CAR T-cells to be resistant to checkpoint inhibition (e.g., PD-1 knockout, dominant-negative PD-1 receptors) can unleash their anti-tumor activity [119].
- TME Re-shaping: Strategies like cyclophosphamide pre-conditioning can rapidly induce pro-inflammatory myeloid and T-cell signatures in tumors, improving CAR T-cell infiltration and anti-tumor immunity [120]. Utilizing inverted cytokine receptors that convert inhibitory signals (e.g., TGF-β) into stimulatory ones (e.g., IL-15) can further enhance CAR T-cell persistence and function within the hostile TME [121].

These approaches collectively aim to dismantle the protective shield provided by the TME, enabling CAR T-cells to effectively reach, persist, and eliminate CSCs.

1.19 Manufacturing and scalability

The manufacturing process for autologous CAR T-cells is complex, time-consuming, and expensive, posing significant challenges for broad clinical accessibility [115]. Delays in production can mean that critically ill patients may progress while awaiting treatment, and the quality of patient-derived T-cells can be compromised by disease state or prior therapies, leading to manufacturing failures or suboptimal products [86] Furthermore, autologous therapies are difficult to scale and can exhibit non-uniformity across patient cohorts [115].

Overcoming manufacturing hurdles is crucial for broad clinical accessibility and cost-effectiveness of CSC-targeted cellular therapies. This points to the practical challenges of translating these advanced treatments. Strategies to address these limitations include:

- Allogeneic Cell Sources: Developing "off-the-shelf" CAR products from healthy donors, such as induced pluripotent stem cells (iPSCs) 37, or allogeneic CAR NK/NKT cells [84]. These sources offer advantages in terms of scalability, reduced manufacturing time, and consistent product quality, as they are not reliant on individual patient cells [122].

- Automated Manufacturing: Implementing semi-automated bioprocessing methods can reduce manual touchpoints, decrease variability, increase efficiency, and improve product quality [123].
- Genetic Engineering Advances: Techniques like CRISPR/Cas9 can enable efficient gene editing for generating alloCAR T-cells with multiple modifications, addressing issues like GvHD and enhancing anti-tumor properties [123].

These efforts aim to make these transformative therapies more accessible, affordable, and consistently effective for a wider patient population.

1.20 Clinical translation and regulatory hurdles

Despite promising preclinical and early-phase clinical trial results, the clinical translation of CSC-targeted therapies, particularly cellular immunotherapies, faces significant regulatory hurdles. Despite promising preclinical and early-phase clinical trial results, the clinical translation of CSC-targeted therapies. Crucially, results from the 2024 trial of Claudin18.2-targeting CAR-T cells in gastric cancer highlighted the potential for targeting specific surface glycoproteins to achieve durable remission in advanced solid tumors [102]. The need for larger, well-controlled trials to confirm safety and efficacy, as well as ensure consistent and durable responses, remains paramount [86]. The heterogeneity in study design and cell product characterization in current trials further complicates clinical adoption [122]. These hurdles are particularly relevant for glycan-targeted therapies, where regulatory frameworks must account for unique bioengineering challenges like glycan-specific affinity tuning.

Rigorous clinical validation and streamlined regulatory processes are essential for bringing these innovative therapies to patients. This highlights the gap between preclinical promise and patient access. Challenges include:

- Data Heterogeneity and Standardization: Lack of standardization across workflows and data collection methods can hinder reproducibility and comparability of results [124].
- Cost and Reimbursement: The high cost of CAR T-cell therapies (e.g., $373,000 to $475,000 per dose) leads to restrictions in insurance coverage and reimbursement, limiting patient access [125].
- Regulatory Frameworks: Navigating diverse and evolving regulatory frameworks across different countries (e.g., Japan) poses additional challenges for global clinical development and approval [126].
- Addressing these challenges requires collaborative efforts among academic institutions, industry, and regulatory bodies to establish standardized protocols, generate robust clinical evidence, and develop sustainable economic models that ensure equitable access to these life-saving therapies.

1.21 Integrated diagnostics for precision oncology

The inherent heterogeneity of cancer, particularly in aggressive forms like neuroblastoma, necessitates a shift towards precision oncology approaches that integrate diverse

diagnostic modalities for comprehensive patient management. Neuroblastoma, the most common extracranial solid tumor in children, is characterized by significant clinical and biological heterogeneity, high relapse rates, and frequent metastasis, making accurate risk stratification and personalized treatment crucial [127].

1.22 Multi-modal diagnostic approaches

Integrating various diagnostic platforms, including liquid biopsy, advanced imaging, and single-cell epigenomic profiling, offers a powerful approach to overcome the limitations of traditional methods and provide a more holistic understanding of tumor biology.

1.23 Liquid biopsy

Liquid biopsy is a minimally invasive technique that analyzes circulating biomarkers in bodily fluids (e.g., blood, urine, cerebrospinal fluid) for real-time monitoring of tumor evolution [127]. It provides valuable information on disease onset, progression, and response to treatment, overcoming the limitations of invasive tissue biopsies which are often difficult to obtain sequentially and may not capture tumor heterogeneity [128]. Key analytes include:

- Circulating tumor DNA (ctDNA) and cell-free DNA (cfDNA): Used for early relapse detection, monitoring MYCN and ALK copy numbers, and identifying actionable mutations [128].
- Circulating tumor cells (CTCs) and CTC clusters: Sensitive non-invasive biomarkers for neuroblastoma diagnosis and prognosis, especially correlating with bone marrow metastasis [129].
- Extracellular vesicles (EVs): Function as reliable cellular surrogates, reflecting global tumor burden and showing potential utility for diagnosis and treatment monitoring in gliomas and neuroblastoma [130].

The clinical detection of CSCs remains challenging due to their rarity. However, the shedding of tumor-associated carbohydrate antigens (TACAs) into the bloodstream offers a specific route for Glyco-based Liquid Biopsy. Circulating tumor cells (CTCs) and exosomes enriched with CSC markers like truncated O-glycans (Tn, STn) or fucosylated CD44 can be isolated using lectin-based microfluidics, offering higher specificity than EpCAM-only capture methods [131]. Furthermore, Immuno-PET (Positron Emission Tomography) utilizing radiolabeled antibodies against GD2 or MUC1 enables the non-invasive visualization of CSC reservoirs and the assessment of glycan-target heterogeneity prior to CAR-T administration [132]. Unlike standard radiomics, glycan-targeted imaging provides a direct readout of the therapeutic target's accessibility.

1.24 Challenges in clinical standardization of glyco-diagnostics

Implementing glycan-targeted diagnostics in routine clinical practice presents unique standardization hurdles. Unlike DNA or proteins, which have template-driven synthesis, glycans are products of complex enzymatic networks sensitive to metabolic fluctuations, leading to batch-to-batch variability in assay results [133].

Mass spectrometry (MS)-based methods are widely regarded as the gold standard for high-resolution structural characterization of glycans, including analysis of released glycans (e.g., via hydrophilic interaction liquid chromatography), intact glycopeptides, and site-specific glycoproteomics [134–136]. However, these approaches are technically demanding, requiring specialized instrumentation, extensive sample preparation, and bioinformatics expertise, which limits their integration into routine hospital workflows due to high costs, time requirements, and the need for dedicated facilities [134–137]. In contrast, simpler methods such as immunohistochemistry (IHC) can detect certain glycoproteins or glycan epitopes (e.g., via lectin staining) but do not provide equivalent detailed structural information on glycan composition or heterogeneity [137]. To realize the potential of precision glyco-oncology, future efforts must focus on developing standardized, automated lectin-arrays or antibody-based ELISA kits that can reliably quantify specific TACA levels (e.g., Tn-MUC1) without requiring specialized MS infrastructure.

These advancements are crucial for realizing the full potential of precision oncology, enabling more targeted and effective treatments, minimizing toxicities, and ultimately improving survival rates and quality of life for cancer patients.

2 Conclusion

The comprehensive review of therapeutic targeting of CSC-specific surface glycans and glycoproteins underscores their pivotal role in driving tumor initiation, progression, metastasis, and, critically, therapeutic resistance. The distinct glycosylation patterns and differential expression of glycoproteins such as CD133, CD44 variants, EpCAM, MUC1, gangliosides (GD2, GD3), and truncated O-glycans present unique vulnerabilities that can be exploited for selective therapeutic intervention.

Current strategies, including monoclonal antibodies and antibody-drug conjugates, demonstrate the capacity to precisely deliver cytotoxic agents or leverage the host immune system against these CSC-specific targets. Cellular immunotherapies, particularly advanced CAR T, NK, and NKT cell designs, offer unprecedented specificity and potency, with ongoing efforts to overcome challenges posed by the immunosuppressive tumor microenvironment, antigen escape, and limitations in manufacturing and persistence. Furthermore, small molecule inhibitors targeting CSC metabolism (metabostemness) and inducing ferroptosis represent innovative approaches to selectively eliminate resistant CSC populations by disrupting their unique metabolic dependencies.

Despite significant progress, challenges persist in achieving absolute specificity, managing tumor heterogeneity and antigen escape, and overcoming the complex immunosuppressive TME. The high cost, manufacturing complexities, and regulatory hurdles also impede broad clinical translation. However, the convergence of multi-modal diagnostics, integrating liquid biopsy, advanced imaging (radiomics), and single-cell epigenomics, holds immense promise for precision oncology. This integrated approach enables real-time, comprehensive molecular profiling of tumors, facilitating accurate risk stratification and guiding personalized treatment strategies, as exemplified in neuroblastoma.

The future of cancer therapy lies in a multi-pronged approach that combines highly specific CSC-targeted therapies with strategies to remodel the tumor microenvironment and leverage advanced diagnostics for dynamic, patient-specific treatment adjustments.

Continued innovation in glyco-engineering, cellular immunotherapy, and AI-driven diagnostic platforms will be essential to realize the full potential of eradicating CSCs, thereby achieving more durable responses and improving the long-term outcomes for patients battling cancer.

Acknowledgements
Not applicable.

Author contributions
Mutaz Jamal Al-khreisat, Waleed K. Abdulsahib, Ihsan Khudhair Jasim, H. Malathi, Priya Priyadarshini Nayak, D. Alex Anand, Gunjan Mukherjee, Aashna Sinha, and Gulsara Ruziyeva contributed to the conception, design, and drafting of the manuscript. All authors read and approved the final version of the manuscript.

Funding
Not applicable.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Faculty of Allied Medical Sciences, Hourani Center for Applied Scientific Research, Al-Ahliyya Amman University, Amman, Jordan
[2]Department of Pharmacology and Toxicology, College of Pharmacy, Al Farahidi University, Baghdad, Iraq
[3]Department of Pharmaceutics, Faculty of Pharmacy, Al-Turath University, Baghdad, Iraq
[4]Department of Biotechnology and Genetics, School of Sciences, JAIN (Deemed to be University), Bangalore, Karnataka, India
[5]Department of Medical Oncology, IMS and SUM Hospital, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar 751003, Odisha, India
[6]Department of Biomedical, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India
[7]University Institute of Biotechnology, Chandigarh University, Mohali, Punjab, India
[8]School of Applied and Life Sciences, Division of Research and Innovation, Uttaranchal University, Dehradun, Uttarakhand, India
[9]Department of Medicine, Termez University of Economics and Service, Termez, Uzbekistan

Received: 29 September 2025 / Accepted: 3 February 2026
Published online: 11 February 2026

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